PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
HARRIET M. STRIMPEL
NEW ENGLAND BIOLABS, INC.
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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	11 AUG 2009
Applicant's or agent's file reference NEB-236-PCT	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US04/39288	22 November 2004 (22.11.2004)	21 November 2003 (21.11.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): C12N 9/16 and US Cl.: 435/199

Applicant

NEW ENGLAND BIOLABS, INC.

1. This opinion contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Kathleen Kerr Telephone No. 571-272-0931

Form PCT/ISA/237 (cover sheet) (January 2004)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/39288

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☒ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☒ in written format

☒ in computer readable form

c. time of filing/furnishing

☒ contained in international application as filed.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 12 and 24

because:

☐ the said international application, or the said claim Nos. _____ relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 12 and 24 are so unclear that no meaningful opinion could be formed *(specify)*:

Improper multiple depdenent claims, see PCT Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 12 , 24

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has:
   - ☐ paid additional fees
   - ☐ paid additional fees under protest
   - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
   - ☐ complied with
   - ☒ not complied with for the following reasons:

   See the lack of unity section of the International Search Report(Form PCT/ISA/210)

4. Consequently, this opinion has been established in respect of the following parts of the international application:
   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-11,13-16,18-20,22,23,26-28 and 31

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-11, 13-16, 18-20, 22-23, 26-28 and 31	YES
	Claims NONE	NO
Inventive step (IS)	Claims 1-11, 13-16, 18-20, 22-23, 26-28 and 31	YES
	Claims NONE	NO
Industrial applicability (IA)	Claims 1-11, 13-16, 18-20, 22-23, 26-28 and 31	YES
	Claims NONE	NO

2. Citations and explanations:

Claims 1-11, 13-16, 18-20, 22-23, 26-28 and 31 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest DNA cleaving enzymes that have at least one mutation *in the beta-bridge separating two catalytic centers* that alters enzyme cleavage activity.

Claims 1-11, 13-16, 18-20, 22-23, 26-28 and 31 meet the criteria set out in PCT Article 33(4), and, thus, have industrial applicability because the subject matter claimed can be made or used in industry. The claimed invention can be used as a reagent in molecular biology.

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made:

Claims 1-11, 13-16, 18-20, 22-23, 26-28 and 31 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because the claims not fully supported by the description. The application, as originally filed, did not describe: the entire genus of modified DNA cleaving enzymes claimed. Only certain species (i.e. T7 endodesoxyribonuclease) with certain mutations (i.e. deletion mutant PA) are described.

Claims 1-11, 13-16, 18-20, 22-23, 26-28 and 31 objected to as lacking clarity under PCT Rule 66.2(a)(v) because of the claims not fully supported by the description. The description does not disclose the claimed invention in a manner sufficiently clear and complete for the claimed invention to be carried out by a person skilled in the art because: it is unclear how to make and use the entire genus of DNA enzymes included in the scope of the claims because the structure of all the DNA enzymes, which relates to their function, is not disclosed.

Claims 1-11, 13-16, 18-20, 22-23, 26-28 and 31 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claims indefinite for the following reason(s): It is unclear what structure (amino acid sequence) is encompassed by the term "T7 Endo I" in claims 1-16 and 26-28. It is unclear what amino acids exactly comprise the beta-bridge in claims 1-16, 18-20, 22-23, 26-28 and 31. Claim 12 is confusing because it depends upon itself (i.e. "according to claim 12"). In claims 16, 19-20, and 22-23, it is unclear what the terms/acronyms "PA" or "PA/A, PA/AA, PA/PGA . . . ." stand for and how to interpret the slash between the acronyms/letters. In claims 26-28, it is unclear what constitutes a single nucleotide polypmorphism (SNP).

CHAPTER I
PCT TELEPHONE MEMORANDUM
FOR
LACK OF UNITY OF INVENTION

PCT No.: PCT/US04/39288

Examiner: Lindsay Odell

Attorney spoken to: Harriet Strimpel

Date of call: 12 July 2005

☐ Amount of payment approved:

☐ Deposit account number to be charged:

☐ Attorney elected to pay for ALL additional inventions

☐ Attorney elected to pay only for the additional inventions covered by

☐ Group(s):

-- encompassing –

☐ Claim(s):

☒ Attorney elected NOT to pay for any additional inventions, therefore, only the first claimed invention (Group I) covered by Claim(s) 1-11,13-16,18-20,22,23,26-28 and 31 has been searched.

☒ Attorney was orally advised that there is no right to protest for any group not paid for.

☒ Attorney was orally advised that any protest must be filed no later than 15 days from the mailing of the Search Report (PCT/ISA/210).

Time Limit For Filing A Protest

Applicant is hereby given 15 days from the mailing date of this Search Report in which to file a protest of the holding of lack of unity of invention. In accordance with PCT Rule 40.2, applicant may protest the holding of lack of unity only with respect to the group(s) paid for.

Detailed Reasons For Holding Lack of Unity of Invention:
Please See Continuation Sheet

Note: A copy of this form must be attached to the Search Report.

ATTACHMENT TO CHAPTER I PCT TELEPHONE MEMORANDUM FOR LACK OF UNITY OF INVENTION

Continuation of Detailed Reasons For Holding Lack of Unity of Invention:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-11, 13-16, 18-20, and 22-23, 26-28 and 31, drawn to a modified DNA cleaving enzyme comprising at least the protein encoded by gene 3 (enterobacteria phage T7/T7 endodeoxyribonuclease I, SEQ ID NO: 1); related nucleic acids, vectors and host cells; methods of expressing said nucleic acids; and a first method of use involving determining whether a DNA substrate has a single nucleotide polymorphism (SNP) by contacting the substrate with at least the enzyme encoded by gene 3.

Group II, claim(s) 1-11, 13-16, 17, 21-23, 31 drawn to a modified DNA cleaving enzyme comprising at least Yersenia pestis phage phiA1122 (SEQ ID NO: 13), related nucleic acids and host cells and methods of expressing said nucleic acids.

Group III, claim(s) 1-11, 13-16, drawn to a modified DNA cleaving enzyme comprising at least Phage Phi Ye03-12 endonuclease.

Group IV, claim(s) 1-11, 13-16, drawn to a modified DNA cleaving enzyme comprising at least Phage T3 endonuclease (phage T3 endodeoxyribonuclease).

Group V, claim(s) 1-11, 13-16, drawn to a modified DNA cleaving enzyme comprising at least Pseudomoas phage gh-1 endonuclease.

Group VI, claim(s) 1-11, 13-16, drawn to a modified DNA cleaving enzyme comprising at least Pseudomoas putida KT2440 endodeoxyribonuclease I.

Group VII, claim(s) 1-11, 13-16, drawn to a modified DNA cleaving enzyme comprising at least Roseophage S101 RP endonuclease I.

Group VIII, claim(s) 25, drawn to a method for modifying enzyme catalytic activity involving selecting an enzyme having two catalytic centers connected by a β-bridge.

Group IX, claim(s) 29, drawn to a method of forming a shotgun cloning library involving incubating a modified DNA cleaving enzyme with a DNA to form non-sequence specific cleavage gragments of the DNA that are ligatable.

Group X, claim(s) 30, drawn to a method for mapping nicks in a duplex DNA involving incubating a modified DNA cleaving enzymes with the duplex DNA in a manganse buffer and permitting nicking to occur.

The inventions listed as Groups I-X do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

The special technical feature of Group I is a DNA cleaving enzyme with at least 35% identity to T7 Endo I (gene 3, enterobacteria phageT7, SEQ ID NO: 1), two catalytic centers separated by a beta-bridge, and at least one mutation in the beta-bridge that effects enzyme cleavage activity. Nucleic acid molecules encoding said enzymes and host cells containing said nucleic acid molecules can also be characterized by this special technical feature. Methods of expressing said nucleic acid molecules are characterized as the first method of making the product of the special technical feature. Methods of

Note: A copy of this form must be attached to the Search Report.

determining whether a DNA substrate has a single nucleotide polymorphism involving contacting the substrate with at least gene 3 are characterized as the first methods of using the product of the special technical feature.

The products of Groups II-VII, drawn to modified DNA cleaving enzymes comprising at least particular polypeptides (i.e. Yersenia pestis phage phiA1122 (SEQ ID NO: 13), Phage PhiYe03-12 endonuclease, etc.) do not share a special technical feature with Group I because they are drawn to enzymes from different organisms/viruses with different structural features (i.e. different amino acid sequences).

Groups VIII-X are drawn to additional methods of making and using modified DNA cleaving enzymes that do not specifically use the special technical feature of Group I (modified T7 Endo I).

Note: A copy of this form must be attached to the Search Report.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under Article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing, the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see the *PCT Applicant's Guide*, Volume II.